CARBONIC ANHYDRASE IN THE KIDNEYS OF EURYHALINE FISH (ANGUILLA ROSTRATA AND SALMO SALAR)

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It has been recognized for nearly 40 years that freshwater fish alkalinize their urine in response to inhibitors of carbonic anhydrase (CA), whereas saltwater fish (both elasmobranchs and teleosts) do not (Maren, Bull. MDIBL 27suppl:28,1987-1988).

In the euryhaline eel (Anguilla rostrata) alkalinization of the urine was observed when freshwater-adapted, but not saltwater-adapted fish, were treated with inhibitor, indicating an absence of renal CA in saltwater-adapted eels. Attempts to measure the renal enzyme however were always thwarted by the presence of blood (which has relatively high levels of CA) and hemopoietic tissue in the kidney (Swenson et al., Bull. MDIBL 14:127,1974). Pure renal tissue has never been obtained from this species.

Our goal is to examine the level of CA activity in purified renal tubules from freshwater- and saltwater-adapted eels, and to compare levels of renal CA mRNA in the respective animals. We hypothesize that freshwater eels will have high CA activity and CA mRNA relative to the saltwater eels.

Eels were trapped in brackish water near MDIBL on Mount Desert Island, ME or obtained from the Huntsman Marine Lab, St. Andrews, Canada. The eels were kept in tanks of running freshwater or saltwater for at least 3 weeks before experiments. To remove blood from the renal vasculature the kidneys were perfused in situ with teleost Ringer's buffer (Wolf, Fish Physiology 6:520,1963) via the dorsal aorta. The perfusion was accomplished by decapitating the eel and inserting the cannula into the dorsal aorta. The caudal portion of the kidney was removed and teased apart to dissociate renal tubules from the hemopoietic cells. The teased tissue was then filtered over 20 micron Nitex, and the tubule fraction remaining on the Nitex was homogenized and used for enzyme assays. The carbonic anhydrase assay is based upon the pH change of barbital buffer in the presence of saturating CO_2 gas, at 0°C.

Despite extensive perfusion, the homogenates were contaminated with hemoglobin as determined by the benzidine test (Sigma Chemical Co.). Whether this hemoglobin is derived from red blood cells remaining in the kidney vasculature or from erythropoietic cells is not known. Based upon the hemoglobin content of the blood, the percentage of blood contamination of the perfused and teased tissues was approximately 1%. Carbonic anhydrase activity, derived from RBCs, in the whole blood of the eels averaged 5000 ± 800 units/ml

(n=6). Therefore, even 1% blood contamination in the tissue will result in approximately 50 units/gm of CA activity. Unfortunately, this is very nearly the activity of enzyme observed in the homogenates, making it difficult to say whether the observed activity was due in part to enzyme from the renal cells, or solely blood/hemopoietic contamination.

Table 1 shows that a large part of the renal enzyme activity in the eel came from the contaminating blood. There is no indication that the freshwater eel has a greater amount of the enzyme than the saltwater eel, or indeed that the enzyme is absent in the saltwater eel kidney, as was surmised from pharmacological evidence.

TABLE I. CA Activity in Renal Homogenates of Euryhaline Fish and Flounder

	Total CA activity	% Blood contamination	CA Activity due to blood	Net CA Activity in renal tissue
FW Eel ± SEM (n=8)	112 ± 40	1.5	77	35 ± 17
SW Eel ± SEM (n=8)	134 ± 38	1.0	54	80 ± 33
FW Salmon (n=2)	462	1.2	134	328
SW Salmon (n=2)	125	1.8	198*	0
Flounder (n=1)	75	2.7	81**	0

Activity of CA in renal tissue is expressed as units/gm tissue as described in Maren et al., Comp. Biochem. Physiol. 67B:69,1980)
* salmon blood CA activity = 11,000 units/ml
** flounder blood CA activity = 3,000 units/ml

Table 1 also shows exploratory work in salmon (Salmo salar)and winter flounder (Pseudopleuronectes americanus). Salmon were obtained from the Penobscot Salmon Company. Some of these fish were from freshwater whereas others had been adapted to saltwater. The results seem to indicate the presence of renal CA in the freshwater salmon, but none in the saltwater salmon. Similarly, the marine winter flounder showed no renal enzyme after correcting for blood contamination.

To test the possibility that some of the carbonic anhydrase activity is associated with the cell membrane (as in the mammalian kidney) Dr. Eva Kinne isolated brush border membranes from eel kidneys. We found no difference in CA activity between the membranes of saltwater- and freshwater-adapted eels; levels being approximately 1 unit/mg protein in each. The significance of this enzyme activity is not clear.

As part of this study we are attempting to detect the presence of CA isozymes in teleosts. In mammals, there are at least 6 isozymes of carbonic anhydrase (CA) which have presumably arisen as a result of gene duplications (Hewett-Emmett et al., Ann. N.Y. Acad. Sci. 429:338,1984). A single form of CA has been identified from the Tiger shark (Bergenhem and Carlsson, Comp. Biochem. Physiol. 95B:205,1990), but there are no DNA sequence data available for any teleost species.

Based upon the physiological function of renal CA in other vertebrates, the most likely CA isozymes to be found in the teleost kidney would be CA-II (a high turnover, cytosolic enzyme) and CA-IV (a high-turnover, membrane-bound enzyme). These two isozymes, working together, are responsible for the reabsorption of filtered bicarbonate from the kidney tubule (Maren et al., Mol. Pharmacol. 44:901,1993).

We conducted Northern blot analyses of eel kidney RNA using mouse CA-II and human CA-IV cDNA probes. The absence of hybridization suggested that the homology between the mammalian and fish nucleotide sequences is not close enough for these probes to be useful (the amino acid sequences of shark and human CA exhibit approximately 60% homology). We therefore decided to generate more specific probes using reverse-transcriptase PCR (RT-PCR). By aligning nucleotide sequences of the different CA isozymes from many species we identified two regions which were conserved among isozymes and species. Oligonucleotide primers were synthesized based upon these conserved regions, and used in the RT-PCR procedure, using freshwater-adapted eel kidney mRNA as substrate. Based upon the CA sequences of other species, we expected a PCR product of approximately 300 base pairs.

When the PCR products were analyzed by electrophoresis there were several bands, one of which was the same size (300 bp) as the fragment generated by PCR of the mouse CA-II and human CA-IV cDNA, which were run as positive controls. The eel PCR fragment was cut from the gel, isolated, and cloned into the pCRII vector (Invitrogen Corp.). We are currently sequencing these clones.

In summary, 1) we were unable to determine the level of true renal carbonic anhydrase activity in eels due to contaminatin by hemopoietic tissue. Further work is planned on improving separation methods, as well as using alternative species in which separation of pure tubular tissue may be feasible. 2) We have identified the presence of presumptive CA mRNA in the eel kidney, and are continuing to characterize the structure and genetic regulation of the enzyme.

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